DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 P24 ANTIGEN
Dupont *Alliance*™
HIV-1 p24 ELISA

I. PRINCIPLE

The Human Immunodeficiency Virus Type 1 (HIV-1) is recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected body fluids or tissues, and from mother to fetus or child during perinatal period. After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigens (Ag) are detectable in blood. In most individuals the antigen level becomes undetectable for a period of time; late in disease, increasing failure of the immune system and increasing levels of virus may again result in detectable levels of antigen. One of the viral components in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1.

The Dupont HIV-1 p24 Antigen Assay is an enzyme immunoassay (EIA, or Enzyme-linked Immunoabsorbant Assay, ELISA) developed for detection and quantitation of the HIV-1 p24 core protein. The Dupont HIV-1 p24 Antigen Assay uses a highly specific mouse monoclonal antibody to HIV-1 p24 antigen coated onto microtiter strip wells. A specimen of plasma, serum or tissue culture medium and lysis buffer are added to a coated well and incubated. If present, the viral antigens bind to the monoclonal antibody on the microtiter well. Following a wash step, biotinylated polyclonal antibody to HIV-1 p24 is added to the well and, during incubation, binds to any HIV-1 p24 antigen bound to the well. Following another wash step, streptavidin-horseradish peroxidase conjugate is added which complexes with biotinylated antibodies. In a final step, a substrate reagent containing orthophenylenediamine-HCL (OPD) is added which reacts with complexed peroxidase to form a yellow color. The reaction is terminated by the addition of acid, and the absorbance is measured spectrophotometrically. The intensity of the color development is directly proportional to the amount of p24 antigen in the plasma, serum or tissue culture medium. The quantity of HIV-1 p24 antigen in a specimen is determined by comparing its absorbance with that of a known HIV-1 p24 antigen standard curve.

II. SPECIMEN REQUIREMENTS

Serum, tissue culture supernatant, or plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin may be used and should be tested as soon as possible following collection. If the situation limits the ability to test the sample quickly, the specimen can be held in refrigeration (2-4^{0}C) for a maximum of 7 days. If the period of time will be greater, the sample can be held at -20^{0}C to -85^{0}C for long-term storage.

Remove the serum from the clot, or plasma from the red cells as soon as possible to avoid hemolysis.

Specimens containing particulate matter may give inconsistent results. Such specimens should be clarified by centrifugation prior to assay.

Heat-inactivated specimens or specimens with obvious microbial contamination are unacceptable.

Avoid subjecting specimens to repeated freeze thaw cycles.

Bring all specimens to room temperature (15-30^{0}C) prior to assay.

III. REAGENTS

A. Reagents included in the Dupont HIV-1 p24 ELISA, 96 (PN 6604534) or 2400 (PN 6607051) kits are:

1. HIV-1 p24 Antibody-coated Microtiter Strips. Store at 2-8^{0}C. Note manufacturer's outdate.

   a. Bring pouch containing HIV-1 p24 antibody coated microtiter strips to room temperature (15-30^{0}C) before opening to avoid condensation on the strips.

   b. The plate consists of 12 removable strips of 8 wells each. Any partial use of strips commits all 8 wells to the assay. Antibody coated strips may be used only once. When using a 96 well plate washer and fewer than 12 strips are needed, place uncoated strips in the remaining positions.

   c. Unused strips may be placed back into the pouch and sealed with the desiccant provided and stored at 2-8^{0}C for 60 days.

2. Detector Antibody (Rabbit polyclonal anti-p24 antibody). Store at 2-8^{0}C. Note manufacturer's outdate.

3. Streptavidin-HRP Diluent. Store at 2-8^{0}C. Note manufacturer's outdate.

4. Streptavidin-HRP Concentrate. Store at 2-8^{0}C. Note manufacturer's outdate.

   a. Within 15 minutes prior to use, prepare Streptavidin-HRP by making a 1:100 dilution of Streptavidin-HRP Concentrate with Streptavidin-HRP Diluent. To prepare the working concentration for a <u>complete 96 well plate</u> add 22 µL of the Streptavidin-HRP Concentrate to 22 mL of Streptavidin-HRP Diluent.

   b. If a partial plate is used, prepare enough Streptavidin-HRP working concentration as shown below:

No of Strips	SA-Buffer (mL)	SA-HRPO (µL)
4	4.0	4
6	6.0	6
12	12.0	12
24	22.0	22

c. Discard unused portion at the end of the day.

5. Substrate Diluent. Store at 2-8^{0}C. Note manufacturer's outdate.

6. OPD Tablets.

a. Within 15 minutes of use prepare sufficient OPD Substrate Solution. With non-metallic forceps or the equivalent, add 1 OPD Tablet to 11 mL of Substrate Diluent for each plate or partial plate assayed.

b. Vortex vigorously to assure complete dissolution.

c. Protect from light. The OPD substrate solution should be colorless to pale yellow. A yellow-orange color indicates that the reagent is contaminated and must be discarded.

d. Discard unused portion at the end of the day.

7. 5% Triton X-100. Store at 2-8^{0}C. Note manufacturer's outdate.

8. Plate Wash Concentrate 20X. Store at 2-8^{0}C. Note manufacturer's outdate.

a. Dilute Plate Wash Concentrate 20X by adding 1 part concentrate to 19 parts distilled, deionized water (i.e., 200 mL Wash Concentrate / 1800 mL dH_2O).

b. Crystals may form in the Plate Wash Concentrate 20X if refrigerated. These should be redissolved by gently warming prior to use.

c. Approximately 1000 mL of diluted (1X) wash buffer is needed per plate assayed. More or less may be needed depending on the type of washer used. Diluted (1X) wash buffer should be prepared fresh prior to use. However, once prepared the diluted (1X) wash buffer has a 1 week expiration date.

9. Stop Solution (4N H_2SO_4). Store at 2-30^{0}C. Note manufacturer's outdate.

B. Reagents required but not provided:

1. 5% Hypochlorite solution (household bleach) diluted 1/100, or appropriate disinfectant.

2. Deionized or distilled water.

3. Standards and controls for the assay provided by the Virology Quality Assurance Laboratory (VQA):

a. VQA SQC (Serum Quality Control). A set of five concentrations. Store at -80^{0}C.

1) Just prior to set up, thaw 1 vial of each of the 5 concentrations.
2) Mix well and use.

IV. SUPPLIES AND EQUIPMENT

Lab coat
Gloves
Micropipet(s) capable of delivering 10-1000 µL volumes
Multichannel pipette(s) capable of delivering 10 µL, 20 µL, 50 µL, 200 µL volumes
Disposable pipette tips suitable for the above pipettes
Disposable reagent reservoirs
Strip holder reaction plate
Serological pipettes
Vortex mixer
Centrifuge
Incubator without CO_2 capable of maintaining 37^{0}C +/- 2^{0}C
Timer capable of measuring times up to 60 minutes
Graduated cylinders and beakers
ELISA microtiter plate washer with waste trap and vacuum source
ELISA microplate plate reader capable of measuring absorbance at 490 or 492 nm with reference at 600 nm

V. PROCEDURE

1. Bring all reagents and samples to room temperature.

2. Create an EIA template in the virology data-management software (see software manual).

3. Position the required number of microtiter strips in the strip holder reaction plate (8 wells per strip). If fewer than 12 strips are needed, use uncoated strip(s) in the remaining positions when using a 96 well plate washer.

4. Add 20 µL of Triton-X to each test well of the coated microtiter plate.

5. Add 200 µL of each VQA SQC concentration and each specimen to the coated microtiter plate according to the template. Cover the plate using an adhesive plate cover.

6. Incubate at 37^0C for 2 hours.

7. Wash as follows: Aspirate the solution from the wells. Add 300 µL of Wash Buffer Working Dilution to each well. Allow wells to soak for 25-30 seconds. Aspirate the solution from the wells. Wash five (5) more times for a total of 6 washes. After the final wash step, grasp the plate <u>firmly</u> along the edges, invert plate over absorbent paper and tap the plate gently to remove any remaining liquid.

   Important: The time between the wash step and the next reagent must be less than five (5) minutes.

8. Add 100 µL of Detector Antibody to all testing wells, <u>except</u> the substrate blank well. Cover the plate using a new adhesive plate cover. Incubate at 37^0C $\pm$ 2 for 1 hour $\pm$ 5 minutes.

9. Wash as described above.

10. Add 100 µL of Streptavidin-HRPO Working Dilution to all testing wells, <u>except</u> the substrate blank well. Cover the plate using a new adhesive plate cover. Incubate at room temperature (15^0C-30^0C) for 30 $\pm$ 5 minutes.

11. Wash as described above.

12. Add 100 µL of freshly prepared OPD-Substrate Solution to all wells. Cover the plate using a new adhesive plate cover. Incubate at room temperature, <u>in the dark</u>, (15-30^0C) for 30 + 5 minutes.

13. Add 100 µL of Stop Solution to all wells.

14. Read the absorbance at 490 or 492 nm, blanking the plate reader on air, (Consult the plate reader Instruction Manual for specific directions) within 15 minutes after

adding Stop Solution. Readings must be taken with a reference filter at >600 nm. Be sure the bottom of the plate is clean and dry prior to reading.

VI. CALCULATIONS

The HIV-1 p24 antigen concentrations may be generated from a virology data-management software program developed for the Division of AIDS (DAIDS) to ensure data integrity of both QA and test specimens. A weighted linear least squares method using the VQA SQC concentrations is used to estimate HIV-1 p24 antigen concentration.

VII. QUALITY CONTROL

The absorbances obtained from the spectrophotometer may be transferred into the virology data-management software program. The software program incorporates two QC check programs, Cum Sum and Levy Jennings. These two programs review the absorbance of the VQA SQC and compare them to established standard deviation ranges. These ranges are determined by the testing laboratory and are reflective of values unique to each laboratory. The software will flag values that fall outside of the laboratory's standard deviation range. The technician must determine the significance of the out of range QC and resolve the situation.

VIII. PROCEDURAL NOTES

Use only reagents from the same kit lot. Do not interchange vials or bottle caps and stoppers.

Plate washing may be automated, semi-automated or manual, but must be carried out with care to ensure optimal performance of the assay. It is recommended that six remove/fill cycles be performed.

IX. REFERENCES

Dupont HIV-1 p24 Antigen Assay package insert and all references within.